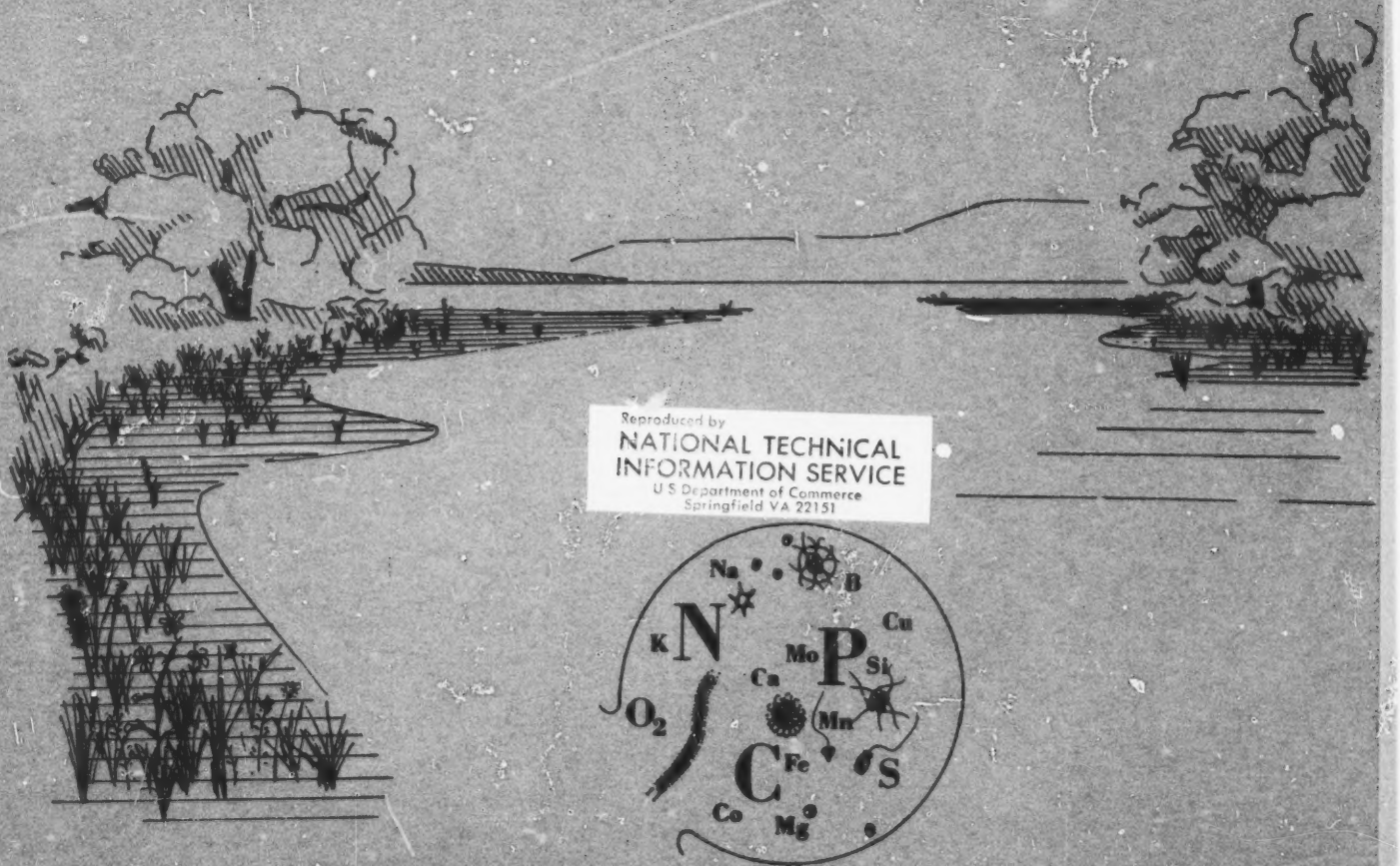
W72-12956

WATER POLLUTION CONTROL RESEARCH SERIES • 16010 EHR 03/72

PB 211 461

ROLE OF BACTERIA IN THE NITROGEN CYCLE IN LAKES



Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

U.S. ENVIRONMENTAL PROTECTION AGENCY

WATER POLLUTION CONTROL RESEARCH SERIES

The Water Pollution Control Research Series describes the results and progress in the control and abatement of pollution in our Nation's waters. They provide a central source of information on the research, development, and demonstration activities in the water research program of the Environmental Protection Agency, through inhouse research and grants and contracts with Federal, State, and local agencies, research institutions, and industrial organizations.

Inquiries pertaining to Water Pollution Control Research Reports should be directed to the Chief, Publications Branch (Water), Research Information Division, R&M, Environmental Protection Agency, Washington, D.C. 20460.

SELECTED WATER RESOURCES ABSTRACTS
INPUT TRANSACTION FORM

1. Report No. | 2. | W 72-12956

ROLE OF BACTERIA IN THE NITROGEN CYCLE IN LAKES,

5. Report Date
6.
8. Performing Organization Report No.

McCoy, E[illegible] F.

10. Project No. EPA Project 16010 EHR 03/72

Wisconsin Univ., Madison, Dept. of Bacteriology

11. Contract/Grant No. ~~16010 EHR 3/72~~

13. Type of Report and Period Covered

12. Sponsoring Organization

Environmental Protection Agency, Water Pollution Control Series, [illegible], March 1972. 23 p. 3 fig., 2 tab., 8 ref.

Biological nitrification as contributing nitrite and nitrate to lakes and streams is explored by determining: nitrifiers, growth sites and activity; denitrifying bacteria numbers and their potential activity as opposing nitrification; field data on nitrite-nitrogen and nitrate-nitrogen in terms of nitrifier and denitrifier populations. Biological nitrification, both heterotrophic and autotrophic, was demonstrated. Values for nitrate-nitrogen above 10 ppm were common; 30 to 60 ppm were found on beaches containing decomposing organic matter. At the same sites denitrifying bacteria were prevalent. More than 70% of 628 samples contained more than 10,000/ml. Even though opposing processes, nitrification and denitrification can coexist in close succession or in adjoining microhabitats. Field values for nitrate-nitrogen and nitrite-nitrogen vary considerably and must be viewed as net values at any given time. Thirteen species of fishes varied greatly in resistance to nitrite-nitrogen. Perch and brook sticklebacks were killed in 3 to 5 hours at 5 ppm. Carp and black bullheads tolerated 40 ppm two weeks and 100 ppm about 24 hours. Susceptibilities of others varied. Nitrite toxicity may influence fish species dominance in a eutrophic lake. (Jones-Wisconsin)

17a. Descriptors

*Bacteria, *Nitrogen cycle, *Lakes, Fungi, Nitrification, Nitrates, Nitrites, Ammonification, Denitrification, Actinomycetes, Nitrogen fixation, Toxicity, Fish, Perches, Carp, Sticklebacks. Bullheads, Suckers, Eutrophication, Frogs, Minnows, Decomposing organic matter, Fry

17b. Identifiers

Autotrophic bacteria, Heterotrophic bacteria

Copy available from GPO Sup Doc EPA 16010 EHR 03/72 $0.35 ; microfiche from NTIS as PB 211 461 , $0.95.

17c. COWRR Field & Group 05C, 02H

19. Security Class. (Report)
20. Security Class. (Page)
21. No. of Pages
22. Price

Send To:
WATER RESOURCES SCIENTIFIC INFORMATION CENTER
U.S. DEPARTMENT OF THE INTERIOR
WASHINGTON, D. C. 20240

E. S. Jones va | Wisconsin Univ., Madison

ia

ROLE OF BACTERIA

IN THE NITROGEN CYCLE IN LAKES

by

Elizabeth F. McCoy
Department of Bacteriology
University of Wisconsin - Madison

for the
Office of Research and Monitoring
ENVIRONMENTAL PROTECTION AGENCY

Program #16010 EHR

March 1972

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C., 20402 - Price 35 cents

EPA Review Notice

This report has been reviewed by the Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

ABSTRACT

In a 3-year study, 1690 samples were tested in the field for NO_3-N, NO_2-N and NH_4-N and in the laboratory for nitrifying and denitrifying bacteria and fungi. The sampling sites were fresh waters, underlying muds and beaches.

Biological nitrification, both heterotrophic and autotrophic, was demonstrated. Values for NO_3-N above 10 ppm were common; 30-60 ppm were often found on beaches with decomposing organic matter.

Denitrifying bacteria were prevalent at the same sites; more than 70% of 628 samples contained more than 10^4/ml. Nitrification and denitrification are opposing processes but can coexist either in close succession or in adjoining microhabitats. Thus, the field values for NO_3-N and NO_2-N vary considerably and must be viewed as net values at any given time.

Experiments with 13 species of locally caught fishes showed great difference in resistance to NO_2-N. Perch and brook sticklebacks were killed in 3-5 hr at 5 ppm. Carp and black bullheads tolerated 40 ppm for 2 wks and 100 ppm for about 24 hr. The susceptibilities of other species varied. Nitrite toxicity may influence the dominance of fish species in a eutrophic lake.

Submitted in fulfillment of Program No. 16010 EHR under sponsorship of the Environmental Protection Agency.

CONTENTS

Section		Page
I	Conclusions	1
II	Recommendations	3
III	Introduction	5
IV	Experimental	7
	Nitrification Studies	7
	Heterotrophic	7
	1969 Field Studies	9
	Denitrification Studies	12
	1970-71 Field Studies	12
	Nitrite Toxicity to Fishes	16
V	Acknowledgments	21
VI	References	23

Preceding page blank

FIGURES

		Page
1	Populations of Denitrifiers in Marsh and Muddy Sites	14
2	Populations of Denitrifiers in Water Sites	15
3	Seasonal Counts of Denitrifiers in Three Sites--Marsh, Stream and Spring	17

TABLES

		Page
1	Spring Survey 1969	10
2	Identity and Characteristics of Fishes	18

SECTION I

CONCLUSIONS

In the course of a 3-year study, 1690 samples were tested in the field for NO_3-N, NO_2-N, and NH_4-N in ppm and in the laboratory for populations of nitrifying and denitrifying bacteria and fungi. The sampling sites were mainly in the Madison, Wisconsin area and included lakes, ponds and streams, their waters, underlying muds and beaches.

1. Biological nitrification, both heterotrophic and autotrophic, was demonstrated by correlating laboratory data for high populations of nitrifiers at sites where field data showed NO_3-N and NO_2-N present.

2. Values for NO_3-N above 10 ppm were common, and concentrations as high as 30-60 ppm were often found on beaches with decomposing organic matter and in muds under shallow water. Water samples from open lakes were generally negative; occasional samples from bays and backwaters were positive at 2 to 3 ppm. Occasional beach sites with piles of dying algae and aquatic weeds in the late summer were positive at 180+ ppm (calculated on the water basis in the beach sand).

3. Denitrifying bacteria, mainly Pseudomonas sp., were prevalent at the same sites, and their numbers ranged from less than 10 to 10^6/ml; more than 70% of 623 samples showed more than 10^4/ml. There was some indication of seasonal fluctuation and of higher denitrifier counts at sites where nitrification was high or had been high previously.

The two processes, nitrification and denitrification, are opposing, but they can coexist either in succession or in adjoining microhabitats at the same sampling site.

4. Toxicity of NO_2-N to fish was shown in the laboratory at levels which are commonly found in the field (formed either by nitrification or by reduction from NO_3-N).

Perch (Percina caproides) were most sensitive, dying in less than 3 hr at 5 ppm; likewise the brook stickleback (Eucalia inconstans) was killed by 5 ppm, but in the slightly longer time of 3-5 hr. On the contrary, the carp (Cyprinus carpio) and the black bullhead (Ictalarus melas) tolerated 40 ppm to the end of the 48-hr test. Even at 100 ppm the carp survived for 45 hr and the bullhead for 24 hr. The common sucker (Catastomus commersoni) lived 48 hr at 100 ppm; and

the quillback (Caproides cyprinus), about 36 hr at 100 ppm. The seven other species tested varied in tolerance, most of them surviving less than 12-24 hr at 20 to 40 ppm.

5. A possible significance of nitrite toxicity is suggested. Since the bottom feeders, such as carp, bullheads and suckers, are most tolerant, their survival in shallow eutrophic waters may be favored. And, conversely, the sensitive fish, such as perch, are killed in a few hours at levels of nitrate which are common, according to our field data for shore samples of eutrophic waters. It is conceivable, therefore, that the effect of NO_2-N may be one factor in the change of dominance of fish species as a lake progresses in eutrophication.

SECTION II

RECOMMENDATIONS

Nitrification and denitrification are opposing processes. Both were shown to be active systems at sampling sites in shallow waters and beaches of lakes and streams.

1. Since the chemical data for NO_3-N, NO_2-N, and NH_4-N for field samples show only the net or temporary balance between nitrification and denitrification (including nitrate reduction), such data are not meaningful alone. Rates of formation and transformation must be obtained for modeling the events of the N cycle in eutrophic lakes.

2. Since nitrification is dependent upon protein N (for heterotrophic) and ammonia N (for autotrophic), nitrification is most active on beaches and in shallow water with dying algae, aquatic plants, or dead fish.

Therefore, such decomposing organic matter should not be allowed to accumulate. Weeds cut from the water should not be piled on shores which drain back to the lake. Masses of dead fish should be removed. Even shoreline improvement by removing fallen trees or other obstructions would help in preventing the accumulation of dead plant material in shallow water.

3. Failure to remove organic matter, as in (2) above, could result in enough NO_2-N (from either the first step of nitrification or from NO_3-N reduction) to cause kills of sensitive fish or their fry in shallow waters. Such NO_2-N, in levels commonly found in the shore sites tested, would be enough to affect the dominance of fish species by favoring carp and other bottom feeders. For these reasons also, shoreline "housekeeping" should be encouraged.

SECTION III

INTRODUCTION

In water, as in soil, the inorganic compounds in the nitrogen cycle are a particularly important nitrogen source for higher plants and algae. Some problems of eutrophication depend upon the rate at which such inorganic nitrogen becomes available, and this rate in turn depends upon bacterial action. Thus, a study of populations of nitrifiers and denitrifiers should reflect potential transformations for which they are the agents.

The objectives of this study were: 1) to explore biological nitrification as contributing nitrite and nitrate to lake and stream waters by determining: a) types and numbers of nitrifiers, and b) their sites of growth and activity; 2) to determine numbers of denitrifying bacteria and their potential activity as opposing nitrification; and 3) to obtain and interpret field data on NO_2-N and NO_3-N in terms of populations of nitrifiers and denitrifiers.

The nitrogen species in question are NH_4-N, NO_2-N and NO_3-N. The processes by which they are formed and transformed and the bacterial agents concerned are as follows:

Ammonification is the release of ammonia by enzymatic degradation of nitrogenous organic matter which is carried out by many heterotrophic bacteria. The ammonia can remain in aqueous solution or be released as gaseous NH_3 to the air. It may later be returned to solution in rain or snow.

Nitrification is the stepwise oxidation from NH_4-N to 1) NO_2-N and 2) NO_3-N, which is carried out by specialized bacteria or fungi; both autotrophic and heterotrophic types are known.

Nitrate reduction is the stepwise reduction of NO_3-N to NO_2-N and usually to NH_4-N. It is the reverse of nitrification. Many bacteria in soil and water carry out nitrate reduction as they metabolize the oxidized N for their own growth or for an electron acceptor. It is important to note that NO_2-N can arise from either step 1 of nitrification or step 1 of nitrate reduction.

Denitrification is the release of gaseous N_2 or N_2O by the reduction of either NO_2-N or NO_3-N. This can be accomplished by relatively few but common types of bacteria in soil and water under conditions when oxygen is otherwise limiting.

Preceding page blank

In view of the above definitions, it is clear that field data for NO_3-N, NO_2-N and NH_4-N do not reveal which process is dominant at the site or time of sampling. Determination of populations of nitrifying and denitrifying bacteria, although not conclusive evidence as to their activity, helps in the interpretation of data.

SECTION IV

EXPERIMENTAL

The work reported here followed several lines which are presented separately, although in some cases the same samples were used for different purposes. The methods are given briefly in each section below.

Nitrification Studies

Heterotrophic

In the past a specialized group of autotrophic bacteria have been considered the main agents of nitrification in soil (Alexander, 1965). Because conditions for their autotrophy are restricted, little attention has been given to excessive nitrification in soil. For the same reasons and, additionally, because of the low level of nitrogen in natural waters of lakes and streams, one would not anticipate strong nitrification in these waters. Yet, high values for NO_3-N in waters do occur at times; and the question arises whether nitrification can account for the levels found, or whether such NO_3-N has its origin external to the lake or stream (e.g., the NO_3-N of fertilizer or soil nitrification under unusual conditions such as runoff from feed lots).

In addition to the autotrophic nitrifiers, certain heterotrophic bacteria, actinomycetes and fungi have now been recognized as nitrifiers (Eylar and Schmidt, 1959; Alexander, 1965). The genera concerned are Mycobacterium, Nocardia, Streptomyces, Micromonospora and Streptosporangium (Hirsch et al., 1961); Arthrobacter (Gunner, 1963); Agrobacterium, Bacillus, Corynebacterium, and Pseudomonas (Alexander, 1965). The first fungus to be recognized as a heterotrophic nitrifier was Aspergillus flavus (Schmidt, 1954, 1963; confirmed by Marshall and Alexander, 1962). It produces nitrate from amino nitrogen in Schmidt's medium. Other soil fungi (e.g., Penicillium sp.) growing in Schmidt's medium cannot form NO_3-N unless provided with NO_2-N, as in step 2 of nitrification. Reviewing the various systems, Alexander (1965) concluded that heterotrophic nitrification occurs only when nitrogen is present in excess of cellular needs and when an energy source other than the oxidation of N is available. Usually heterotrophic nitrification by bacteria in Amino N medium stops at NO_2-N formation, although NO_3-N can subsequently be formed by the Penicillium type system using NO_2-N

as substrate for NO_3-N formation. It may be noted, too, that autotrophic nitrifiers could carry out this final oxidation.

Since many of these heterotrophic nitrifiers are common in waters of lakes and streams and in the adjoining beaches and soils of the immediate drainage basin, this investigation was undertaken to assay their importance as contributors of nitrate and nitrite to eutrophic waters.

Confirmation of heterotrophic nitrification by fungi. As a starting point, the work of Schmidt with A. flavus was confirmed and his technique was then adapted to the testing of water samples. The medium of Schmidt is:

Part A	K_2HPO_4.....	1.0 g
	$MgSO_4 \cdot 7H_2O$.....	0.5
	$FeSO_4 \cdot 7H_2O$.....	0.01
	$MnSO_4 \cdot 4H_2O$.....	0.01
	Distilled water..	900 ml
Part B	Glucose.....	4.0 g
	Peptone.....	8.0
	Distilled water..	100 ml

The parts are sterilized separately and combined in the desired quantity as needed. In the experiments reported below, 100 ml in 500 ml Erlenmeyer flasks were used.

A. flavus was tested along with a collection of 128 other species and strains, kindly provided by Dr. Kenneth Raper, Bacteriology Department, University of Wisconsin. They comprised all 8 morphological groups within the genus Aspergillus. They were grown in Schmidt's medium on a rotary shaker at 30°C for 5 days (aerated vigorously) or in standing cultures at 30°C for 14 days (aerated gently). Concentrations of NO_3-N, NO_2-N and NH_4-N were determined by the Bremner-Keeney distillation method (Bremner and Keeney, 1965).

Approximately 70% of the cultures tested produced NO_3-N in amounts ranging 28-115 µg/ml. The highest producers were the A. flavus and A. wentii groups; all strains produced 65-100 µg/ml. Of 24 species and strains of Penicillium, 21 were able to produce NO_3-N at 5-10 µg/ml levels but only when the amino N of Schmidt's medium was supplemented with NO_2-N. Similarly, several cultures of Fusarium, Gliocladium, Memnoniella, Scopulariopsis and Myrothecium produced 9-13 µg/ml of NO_3-N

when supplied with NO_2-N. These latter genera have not been recognized before as nitrifiers.

1969 Field Studies

The study was begun by comparing heterotrophic vs autotrophic activities of cultures in 2 series: Amino N medium of Schmidt for the heterotrophic and NH_4-N Synthetic medium for the autotrophic. The latter medium was chosen from the general literature on nitrification by soil autotrophs; it contained:

Glucose.....	2.0 g
$(NH_4)_2SO_4$.....	1.0
$MgSO_4 \cdot 7H_2O$.....	0.2
$CaCl_2 \cdot 2H_2O$.....	0.1
$FeSO_4 \cdot 7H_2O$.....	0.01
$ZnSO_4 \cdot 7H_2O$.....	0.001
$MnSO_4 \cdot H_2O$.....	0.001
$Na_2MoO_4 \cdot 2H_2O$.....	0.001
Distilled water..	1000 ml

Again the glucose and mineral salts were separately sterilized and combined to give 100 ml of medium in 500 ml Erlenmeyer flasks. Incubation was at 30°C in shaken and standing cultures for 14 days.

In the spring of 1969, 191 samples were taken of waters, underlying muds and beach sands from lakes, ponds and shallow streams. On the larger lakes (Monona and Mendota) various shore conditions were represented. Most of the samples were obtained from the Madison area, but, in addition, 3 came from the Wisconsin River, 6 from the Sioux River and 6 from Lake Superior. As shown in Table 1, the greatest number of positives appeared in Schmidt's medium. Microscopic examination showed numerous gram-negative bacteria, actinomycetes and vegetative fungal hyphae, which indicates active heterotrophic nitrification. Forty-seven of these cultures were retested in Schmidt's medium; although still only crude enrichment cultures, 45 of them produced NO_2-N in the range of 2-154 µg/ml, with an average of 48 µg/ml. Only 2 of them produced NO_3-N (33 and 46 µg/ml). These 2 cultures contained numerous fungal hyphae but the genus is unknown, since only vegetative growth was seen. They could have been Aspergillus, probably not Penicillium sp. Thus, it appears that for these 191 samples the heterotrophic process of nitrification was dominant and that most of the action stopped at NO_2-N. How-

ever, this is not conclusive evidence for lack of autotrophic nitrification because of the difficulty in quantitative assays for the autotrophic nitrifiers. Nevertheless, the autotrophic system, if active, should have proceeded to NO_3-N. In addition, the conditions in Schmidt's medium favor the conclusion that the heterotrophic system was present and presumably active under the conditions of the test.

Table 1

Spring Survey 1969. Testing of 191 samples for nitrification in laboratory media, differential for autotrophic and heterotrophic systems.

	Positive samples in media	
	NH_4N	Schmidt's
Samples tested	(autotrophic)	(heterotrophic)
Waters - 32	1	4
Shallow mud of lakes and ponds - 25	5	11
Beaches - 38	1	28
Stream mud and sand - 23	17	15

"Positive" means any value in excess of minimum recorded, >2 µg/ml for NO_2-N and >5 µg/ml for NO_3-N.

In the summer of 1969, a more extended survey was made, involving 700 samples taken from 66 stations in the Madison lakes area. The sampling continued over a 3-1/2 mo period, and most of the stations were visited from 6-10 times. Records of the weather, water temperature and biota at the stations were kept, and the NH_4-N, NO_2-N and NO_3-N tests were done in the field. For the latter purpose, a field kit and spot plate test were developed, using Nessler, dimethyl α-naphthylamine and diphenylamine reagents for ammonia, nitrite and nitrate respectively. Known standards of $(NH_4)_2SO_4$, $NaNO_2$, and $NaNO_3$ at 100 ppm as NH_4-N, NO_2-N and NO_3-N were carried in the kit. Proper dilutions were made in the field, and color comparisons were made between the test samples and known standards. An example of the procedure and sensitivity of matching in the field is as follows. If the sample being tested for NO_3-N showed a distinct but light blue color, it would be compared with 0,1,2,5 ppm of the known standard. If dark or maximum blue

color was seen in the first spot test, the sample would be diluted to match the color for 2 ppm and the dilution factor used to calculate the NO_3-N value for the sample. The colors for "trace" and 1 ppm of the known standard were appreciable, but the readings for less than 2 ppm were rather subjective. Thus, such values were reported as less than 2 ppm without differentiation. All higher values are reported as calculated data.

Water samples from open lakes and streams were generally negative (no color by our spot test); occasional samples from bays and backwaters were positive at less than 2 to 3 ppm as NO_3-N. The data for shore samples were sorted into 2 lists: those greater and less than 10 ppm. In general, values less than 10 ppm resulted from samples collected on clean sandy shores. Water weeds and algae were identified and their presence at the shore sites recorded, because of epiphytic bacteria on them. Beach samples, particularly those taken under masses of dying algae, Myriophyllum or Lemna, were nearly always positive at levels greater than 10 ppm. Occasionally such samples in late summer showed 180-200 ppm of NO_3-N (calculated on the water basis in the sandy beach sample). Most such beach samples, however, showed 30-60 ppm of NO_3-N on the same basis. Ammonia was high but the amount varied greatly. In one mass of dying algae, an ammonia concentration of 600 ppm was found and the pH was understandably high--pH 9-9.8. Thus, at certain places at least, there is substrate N for both heterotrophic (dead algal N) and autotrophic (NH_4-N) nitrification.

About one half of these samples were also tested in the laboratory in shaken flasks at 30°C, to determine their potential to support higher nitrification under aerated conditions. Most of them (236 samples) did support higher nitrification. They became strongly positive, but still yielded less than 10 ppm after 10 days. Many of these samples, when tested in the field, showed very low NH_4-N values, ranging from trace amounts to 5 ppm. Thus, their nitrification potential was apparently limited by lack of N. Consequently, another set of 82 samples taken at random were supplemented with 50 ppm ammonia as $(NH_4)_2HPO_4$ and incubated on a rotary shaker at 30°C. Within five days 79 of the 82 became positive for NO_3-N with an average of 6.2 ppm. Longer incubations were not tested. Since no attempt was made to provide optimum conditions for either heterotrophic or autotrophic nitrification, it cannot be said how high the values might have been. It is remarkable that NO_3-N was produced so rapidly, and this appears to mean that autotrophic nitrifiers were present and were able to respond immediately to NH_4-N added to the low-nutrient natural water samples.

A further indication that available N is the determining factor in nitrifying activity can be seen in the following laboratory observation. Six samples of Lake Mendota Bay mud and related water were set up in aquaria and stocked with small minnows. One aquarium initially had no detectable NO_3-N and was still negative at 30 days. All five others were positive at levels greater than 10 ppm at 30 days, but the time at which they became positive was quite variable. One that was low (2 ppm at 30 days) was then supplemented with 50 ppm of NH_4-N as $(NH_4)_2HPO_4$ and became positive--6 ppm at 10 days, 22 ppm at 30 days.

In the course of use of some of the aquaria, the experimental fishes died. One of them was removed to a beaker with 100 ml of Lake Mendota water and allowed to decompose naturally (by proteolysis and ammonification). After 5 days, the remains of the fish and all floating debris were removed and the water aerated at room temperature on a magnetic stirrer. A control of the same water without the fish autolysate was run in parallel, and it remained negative for nitrification at 20 days. The fish-water specimen became positive at 8 ppm by 5 days and greater than 20 ppm by 20 days. It was noted in the summer survey that beach sand samples taken under dead fish often were strongly positive in the 30-to-greater-than-60 ppm range.

Denitrification Studies

1970-71 Field Studies

A small survey was made during June and early July to confirm the results of the previous summer. A total of 175 samples were taken from the same sites as in 1969, and 25 additional samples were collected from new sites on lake shores and small streams. The results showed that high NO_2-N values (2 to 18 ppm) and less commonly high NO_3-N values (greater than 10 to as high as 70 ppm) were related to organic matter on the shore sites. However, in some cases it was found that repetition of sampling at the same site on successive days did not always confirm the previous high value. Sometimes a storm or visible change, such as wave action at the site, seemed to account for the difference, but this was not always the case. Thus, it was decided that denitrification should be studied as the main program for the summer of 1970, and the results were so interesting that an extension of time for study in the summer of 1971 was sought. The discussion which follows presents the results of both summers of study.

During late August to December 1970, 245 samples were tested simultaneously for both nitrification and denitrification

activity. These samples were collected from Lakes Mendota, Monona, Waubesa and the Nine Spring Creek. The nitrification tests consisted of field determinations as before, and the results were consistent with the prior data. The denitrification activity was judged from dilution counts on Giltay's medium (mineral salts with nitrate as the N source and citrate as the C source). Readings were taken on the basis of gas, N_2 or N_2O, and MPNs were calculated. The denitrifiers at the different sites ranged from less than 10 to more than 5×10^6/ml; 189 of the 245 samples had more than 10^3/ml and 144 of them had more than 5×10^4/ml. From the high dilution gas-positive tubes, 3 to 5 serial transfers on Giltay's medium were made to provide enrichments from which to isolate and identify the denitrifiers. Over 85% of the 300+ cultures tested proved to be Pseudomonas sp. This was not unexpected, since pseudomonads are the principal denitrifiers in the soil and are numerous in all natural waters.

An analysis of the total body of data on denitrification was made. This included the 245 samples of the summer of 1970, followed by 378 samples taken mainly during the winter and spring of 1970-71 and a few collected during the months of July and August of 1971. The samples were sorted according to the following types of sites:

Open lake waters
Stream and spring waters
Shore waters--turbid, suspended matter both living and dead
Runoff waters
Beach waters--clear, off sandy beaches

Marsh muds, usually marl
Muds under shallow water
Sand on beaches, with small organic debris
Dry weeds and algae on beaches

The data pertaining to these sites are grouped as to waters and muds in Figures 1 and 2. The graphs show numbers of samples positive at each population level of denitrifiers detected. These graphs are not based upon equal numbers of samples, and in a few cases only a few samples were tested, but nevertheless they do show several interesting points, namely: 1) the great range of numbers of denitrifiers from less than 10 to 5×10^6/ml. Commonly, 10^4 to 10^5/ml were found regardless of whether the specimens were mud or water; 2) water samples yielded a greater number of positives than mud samples; and 3) stream and spring water samples, and shore water samples provided the largest number of positive

Figure 1. Populations of denitrifiers in marsh and muddy sites

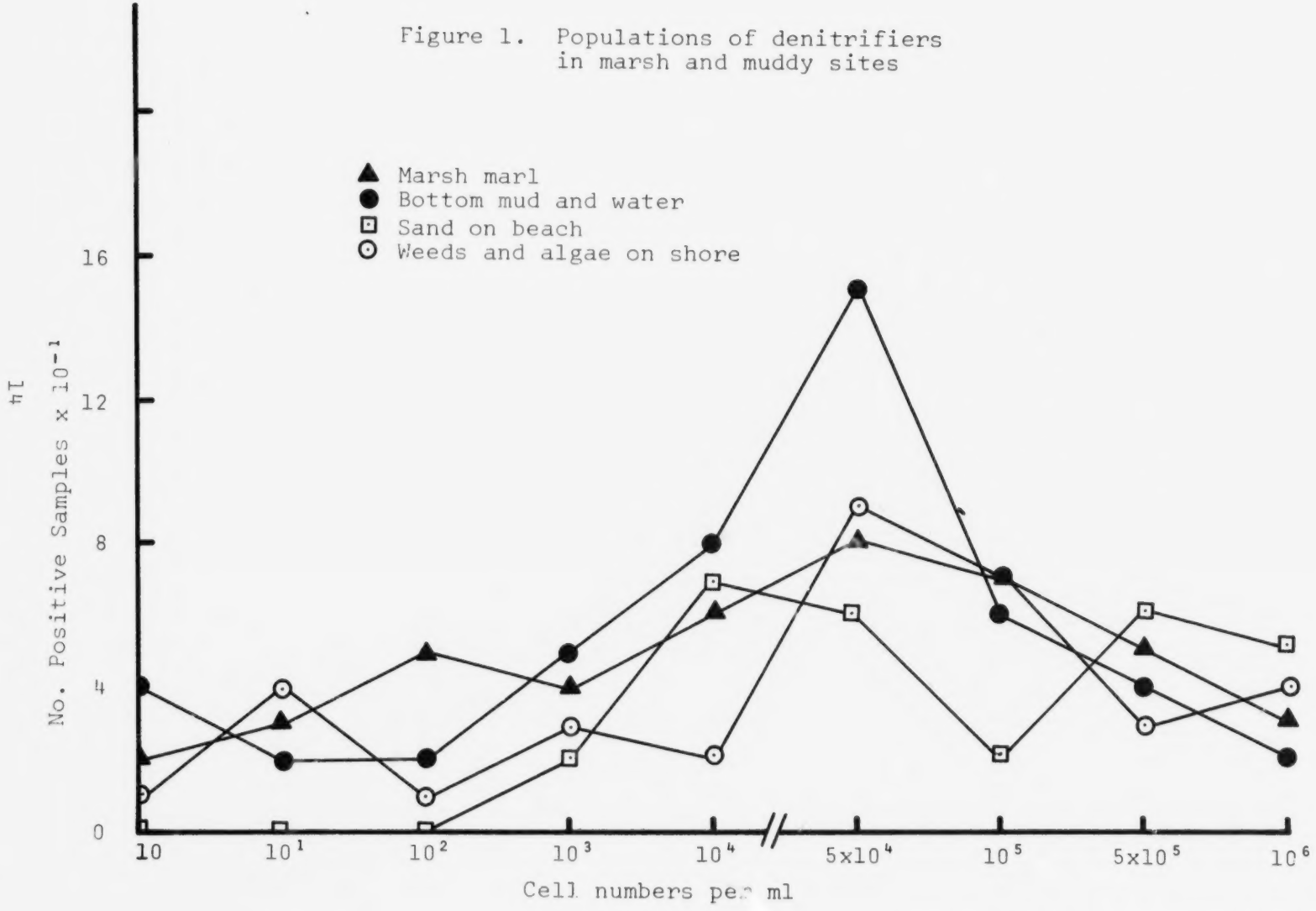
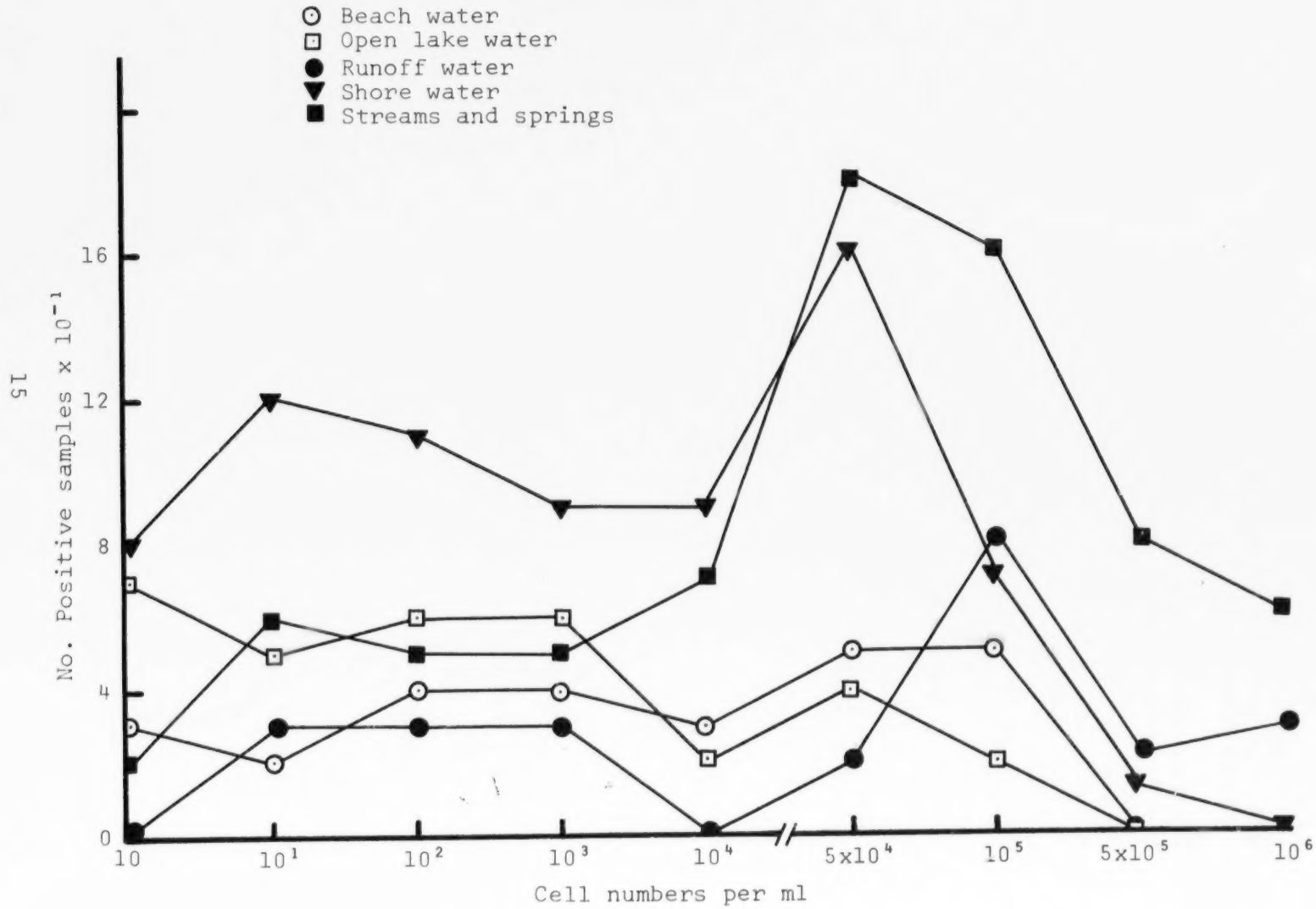


Figure 2. Populations of denitrifiers in water sites



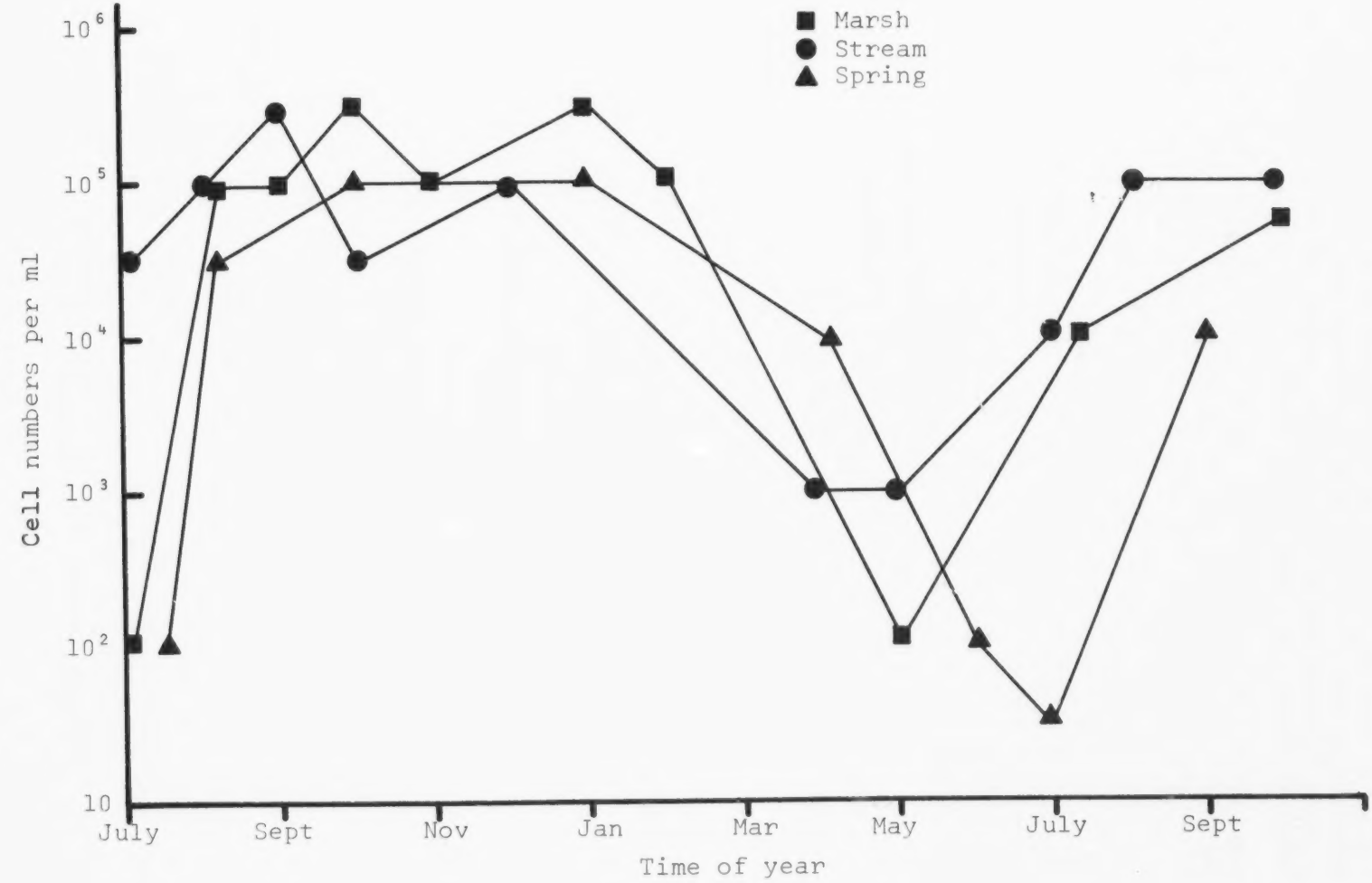
samples. This latter point is not a reflection of greater numbers of such samples; apparently these sites are favorable for growth of pseudomonads, but the controlling factors are not known.

Another approach to analyzing the ecology of the denitrifiers was made by plotting the numbers/ml against seasons of year. Figure 3 is a graph of data for 3 types of sites: streams, springs and marshes. The populations of denitrifiers are high in winter months, drop in late spring, and rise again in late summer. The rise in late summer was confirmed by the data from the second summer. The peak in winter is understandable because of cold-weather survival of the late fall populations. The drop in spring is more difficult to explain. It may be due to a low point in available NO_3-N substrate as water plants absorb their nitrogen. Another possibility is antagonism of the general microbial flora in the warming waters. Perhaps, readily available nutrients become limiting, but this is not likely because of the diverse substrates utilizable by pseudomonads. Possibly it is a combination of factors, such as lack of nitrate substrate in late spring, which prevents the aerobic pseudomonads from competing with the more facultative and fermentative bacteria of the lake. Conversely, in winter under the ice where free oxygen may become limiting to the general bacteria, the nitrate-reducing denitrifiers would have an oxygen advantage over many bacteria. There could then be growth of denitrifiers to account for the high winter populations. This is possible, because many pseudomonads are psychrophiles. Unfortunately, we did not test the denitrifying pseudomonads for growth at near freezing temperatures. All of the above comments are speculative but could account for the apparent seasonal changes in the populations of denitrifiers. High populations and potential activity of denitrifiers are important because of the possible opposition to nitrification in natural water systems.

Nitrite Toxicity to Fishes

It was recognized that concentrations of both NO_3-N and NO_2-N in some samples were within the known levels for toxicity to animals, and perhaps also to plants. Such toxicity could potentially have considerable importance in eutrophic lakes. The death of frypan and game fishes in certain shore sites could be considerable and, if differences in susceptibility could be shown, toxicity might be a factor in the change of species dominance in eutrophic lakes.

Figure 3. Seasonal counts of denitrifiers in three sites: marsh, stream, and spring



Nitrite toxicity was studied first, because it is the more toxic ion and, if the concentrations found in lake and stream systems were not toxic to fish, there would be no need to test the less toxic NO_3-N.

Representatives of 13 species of fishes were used. The species tested, their preferred habitats and food, and where they were caught in the Madison area are given in Table 2.

Table 2

Identity and characteristics of the fishes

Percidae

Percina caproides Log perch

Collected from L Mendota and Mississippi R

On rocky shores, feeding on crustaceans, tubefix and various invertebrates

Etheostoma nigrum Johnny darter

Collected from L Mendota but usually in streams

Feeding like perch

Centrachidae

Lepomis macrochirus Bluegill

Collected in slow and stagnant waters, feeding on invertebrates in surface water

Lepomis gibbosus Pumpkinseed

Like bluegill but preferring many molluscs, esp snails, in diet

Cyprinidae

Netropis spilopterus Spotfin shiner

Pelagic, spawning in rivers

Plankton feeders

Netropis stramineus Sand shiner

From Wisconsin R at Spring Green; in open sandy areas of rivers

Plankton feeders

Cyprinus carpio Carp

Collected from Sugar R and Black Earth Cr

Bottom feeders

Table 2 cont.

Gasterosteidae

Eucalis inconstans Brook stickleback

Collected from Dunn's marsh, in weed-clogged channels

Voracious feeder on live food only, but after feeding often kills all remaining live food in sight; rather inactive between feedings

Catastomidae

Hypentilium nigricans Hog sucker

Collected from Black Earth Cr

Bottom feeder on aquatic plants and detritus

Catastomus commersoni Common white sucker

Collected from L Mendota

Bottom feeder on detritus, often in polluted water

Caproides cyprinus Quillback

Collected at Wisconsin R

Bottom feeders in sandy silt

Ictaluridae

Ictalurus melas Black bullhead

Collected in L Waubesa, Hog Island

Bottom feeders, predacious on molluscs, fish, invertebrates, also saprophytic feeders on detritus

Noturus flavus Stone cat

Collected in Black Earth Cr

Feeding in rocky-riffle areas on drifting matter

The fish were held in the laboratory in Madison city water in well aerated aquaria until tested, usually within 2-3 days of capture. Toxicity tests were conducted with 3 small fingerlings or minnows of the test species in gallon jars. The sides of the glass jars were painted to minimize disturbances during the test period. The levels of NO_2-N tested were 5,10,20,40,100,200 ppm, a range covering the NO_2-N values actually found in the field (max 73 ppm) or potentially found in aerated lake water in the laboratory (max 154 ppm). As stated earlier, 30-60 ppm of NO_3-N (readily reducible to NO_2-N) is the range more commonly found at shore sites with decomposing organic matter. The range from 30 to 45 ppm of NO_3-N in water is generally regarded as potentially toxic to some animals.

Great differences were found in the susceptibility of fishes to NO_2-N. Perch (Percina caproides) were most sensitive, dying in less than 3 hr with 5 ppm. Likewise, the brook stickleback (Eucalia inconstans) was killed by 5 ppm, but in the slightly longer time of 3 to 5 hr. On the contrary, the carp (Cyprinus carpio) and the black bullhead (Ictalarus melas) tolerated 40 ppm to the end of the 48-hr test. Even at 100 ppm the carp lived for about 45 hr and the black bullhead 24 hr. The common sucker (Catastomus commersoni) lived 48 hr at 100 ppm, and the quillback (Caproides cyprinus) about 36 hr. The other species tested ranged between these extremes, most of them surviving less than 12-24 hr with NO_2-N in the range 20 to 40 ppm. Control fishes were kept under the same conditions except for the NO_2-N challenge, and they were living and fully active after 4 weeks.

The green frog (only one tested) was remarkably resistant. It lived 4 weeks in water with added NO_2-N at 100 ppm; in fact, the NO_2-N was added several times to make up for losses by microbial action. Finally, when subjected to a challenge dose of 200 ppm, the frog died within 22 hr.

The differences in susceptibility of the fishes cannot now be explained. It is interesting that the bottom feeders were the most resistant, and this fact may have some bearing upon their ability to survive and displace the more desirable species in eutrophic waters. It is also interesting that the perch and several species of minnows were susceptible to as low as 5 ppm NO_2-N, and our field data show that this range of concentration is common in shore samples from eutrophic lakes. It is conceivable that nitrite toxicity could be very serious to the fry in their shoreline habitat.

SECTION V

ACKNOWLEDGMENTS

It is a pleasure to acknowledge support of this work by Program 16010 EHR of the Environmental Protection Agency. The stimulus received from Dr. Paul Uttormark and other colleagues in the eutrophication program in the past three years has been very beneficial. Thanks are also due the graduate student assistants, Margaret Heimbrook, Terry Thompson and James Kenyon, who participated in the field and analytical work, and to Don Samuelson for his aid in obtaining the fish and testing nitrite toxicity upon them.

SECTION VI

REFERENCES

Alexander, M. 1965. Nitrification. Chapter 8 in Soil Nitrogen, Bartholomew, W. V., and F. E. Clark, eds. Amer. Soc. Agron., Inc., Madison, Wis.

Bremner, J. M., and D. R. Keeney. 1965. Steam distillation methods for determination of ammonium, nitrate and nitrite. Anal. Chim. Acta 32:485-495.

Eylar, O. R., Jr., and E. L. Schmidt. 1959. A survey of heterotrophic microorganisms from the soil for ability to form nitrite and nitrate. J. Gen. Microbiol. 20: 473-481.

Gunner, H. B. 1963. Nitrification by Arthrobacter globiformis. Nature 197:1127-1128.

Hirsch, P., L. Overrein, and M. Alexander. 1961. Formation of nitrite and nitrate by actinomycetes and fungi. J. Bacteriol. 82:442-448.

Marshall, K. C., and M. Alexander. 1962. Nitrification by Aspergillus flavus. J. Bacteriol. 83:572-578.

Schmidt, E. L. 1954. Nitrate formation by a soil fungus. Science 119:187-189.

Schmidt, E. L. 1963. Cultural conditions influencing nitrate formation by Aspergillus flavus. J. Bacteriol. 79:553-557.

U.S. GOVERNMENT PRINTING OFFICE 1972—484-466/247

SELECTED WATER RESOURCES ABSTRACTS
INPUT TRANSACTION FORM

1. Report No.
2.
3. Accession No. **W**

4. Title: ROLE OF BACTERIA IN THE NITROGEN CYCLE IN LAKES

5. Report Date
6.
8. Performing Organization Report No.

7. Author(s): McCoy, Elizabeth F.

10. Project No.: 16010 EHR

9. Organization: University of Wisconsin, Madison
Water Resources Center

11. Contract/Grant No.

13. Type of Report and Period Covered

12. Sponsoring Organization

15. Supplementary Notes

16. Abstract In a 3-year study, 1690 samples were tested in the field for NO3-N, NO2-N and NH4-N and in the laboratory for nitrifying and denitrifying bacteria and fungi. The sampling sites were fresh waters, underlying muds and beaches.

Biological nitrification, both heterotrophic and autotrophic, was demonstrated. Values for NO3-N above 10 ppm were common; 30-60 ppm were often found on beaches with decomposing organic matter.

Denitrifying bacteria were prevalent at the same sites; more than 70% of 628 samples contained more than 10,000/ml. Nitrification and denitrification are opposing processes but can coexist either in close succession or in adjoining microhabitats. Thus, the field values for NO3-N and NO2-N vary considerably and must be viewed as net values at any given time.

Experiments with 13 species of locally caught fishes showed great differences in resistance to NO2-N. Perch and brook sticklebacks were killed in 3-5 hr at 5 ppm. Carp and black bullheads tolerated 40 ppm for 2 wks and 100 ppm for about 24 hr. The susceptibilities of other species varied. Nitrite toxicity may influence the dominance of fish species in a eutrophic lake.

17a. Descriptors
*Ammonification, *Aquatic bacteria, *Aquatic fungi, *Denitrification, *Nitrate reduction, *Nitrification, *Nitrite toxicity, *Nitrogen compounds, *Nitrogen cycle, *Nitrogen fixation, Ammonia, Decomposing organic matter, Eutrophication, Nitrates, Nitrites, Toxicity.

17b. Identifiers
Autotrophic bacteria, Heterotrophic bacteria.

17c. COWRR Field & Group 05C

18. Availability
19. Security Class. (Report)
20. Security Class. (Page)
21. No. of Pages
22. Price

Send To:
WATER RESOURCES SCIENTIFIC INFORMATION CENTER
U.S. DEPARTMENT OF THE INTERIOR
WASHINGTON, D. C. 20240

Abstractor Elizabeth McCoy | Institution University of Wisconsin-Madison